Quit Stalling or You'll Be Silenced

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The use of small RNAs to program gene regulation and genome defense necessitates ever-changing choices about the sequences used for small-RNA biogenesis. Dumesic et al. now reveal stalled spliceosomes as a trigger for small-RNA biogenesis in the pathogenic fungus *Cryptococcus neoformans*.

A striking feature of RNA-based silencing is its adaptability. In most eukaryotes, a silencing "hardware" that includes Argonaute proteins and other silencing factors stands ready to act, but on its own, it lacks the information needed to identify appropriate regulatory or invasive RNA targets. Various categories of ~20–30 nucleotide (nt) RNAs, including short interfering RNAs (siRNAs), microRNAs (miRNAs), and piwi-interacting RNAs (piRNAs), constitute the "software" that instructs this silencing machinery about its targets. By using a system that is programmable rather than hardwired, cells can respond to ever-changing regulatory needs on the fly, simply by changing the pool of sequences that feed into the small-RNA biogenesis pathways. Once the basic hardware-software framework was established, natural selection could identify many different small-RNA biogenesis criteria that provide adaptive value, the classic example being extensively double-stranded character. In this issue of *Cell*, Dumesic et al. (2013) now reveal stalled spliceosomes, the ribonucleoprotein complexes that remove introns from pre-messenger RNAs (pre-mRNAs), as a previously unappreciated source of small silencing RNAs in the opportunistic fungal pathogen *Cryptococcus neoformans*.

Previous analyses established the presence of functional RNA silencing pathways in *C. neoformans* and implicated orthologs of known silencing factors, including an RNA-dependent RNA polymerase (Rdp1), two Dicers (Dcr1/2), and an Argonaute (Ago1) (Wang et al., 2010). This study yielded an interesting twist—silencing efficacy increases dramatically during mating and meiosis, a phenomenon dubbed "sex-induced silencing." However, silencing is still apparent during mitotic growth (Wang et al., 2010, 2012), and Dumesic et al. (2013) began their examination of *C. neoformans* siRNA biogenesis by profiling the small-RNA population in vegetatively growing haploid cells. Intriguingly, a majority of the reads were antisense to transposon or transposon-like centromeric sequences with apparently suboptimal introns. Their data set included reads that spanned intron/exon junctions, suggesting origins related to pre-mRNAs and, by inference, the nucleus.

To pursue this connection further, Dumesic et al. (2013) conducted a series of coimmunoprecipitation, candidate gene inactivation, two-hybrid, and fluorescence microscopic analyses and identified a protein complex they have termed Spliceosome-Coupled And Nuclear RNAi (SCANR). This complex is composed of Rdp1, Ago1, and three additional proteins they have named Qip1, Gwc1, and Srr1. This last protein provided another unexpected link between small RNAs and introns, given its similar domain structure to SR proteins, which are well-known pre-mRNA splicing regulators (Shepard and Hertel, 2009).

Could these correlative and physical links reflect functional connections? Yes—removing introns from an otherwise robust siRNA-generating locus led to a dramatic decrease in siRNA production from that gene. Conversely, when the authors used splice-site mutations to compromise the splicing of a non-small-RNA-generating gene, it became a much more efficient source of small RNAs. Tellingly, the latter effect depended on the nature of the splicing block. 3′ splice-site mutant pre-mRNAs, which assemble into spliceosomes but stall before the second catalytic step, exhibited a strong increase in siRNA production. By contrast, 5′ splice-site mutations, which bypass the splicing pathway, did not, suggesting a specific role for the SCANR complex's physical engagement with the spliceosome.

Exploring the mechanism further (Figure 1), the authors found that deletion of the gene *DBR1*, which encodes the debranching enzyme that linearizes excised intron lariats or stalled spliceosomal intermediates destined for degradation, completely blocked the production of these siRNAs. Based on these results, Dumesic et al. (2013) propose that a kinetic competition between splicing and siRNA production operates during mRNA biogenesis. Suboptimally spliced introns exhibit a greater dwell time on spliceosomes, and pre-mRNAs in these stalled spliceosomes are substrates for the synthesis of siRNAs that will be used to dampen expression from the same intron-containing loci.

What could be the functional rationale for linking stalled spliceosomes to siRNA production? Dumesic et al. (2013) argue that this connection gives the RNAi pathway another entrée into transposon silencing, which is a well-established natural role for small-RNA pathways in many species (Malone and Hannon, 2009). The molecular logic here is that, when a foreign genetic element invades a cell, its introns are unlikely to arrive preoptimized for that species' splicing machinery and that the resulting stalled spliceosomes constitute an effective reflection of "non-self" status. The specific genes examined in this paper include

some that are annotated as transposon-like, but the proposed role for the SCANR pathway in limiting transposition as such awaits further direct tests. Given the selfish nature of transposons, this model predicts the existence of strong selective pressure toward either intron loss or intron optimization in transposon-encoded genes in *C. neoformans*, but these predictions are not borne out in the data of Dumesic et al. (2013). They suggest two nonexclusive explanations. First, some transposons may have simply invaded too recently for such pressure to have acted. Second, they note that splice sites near transposon termini can minimize the deleterious effects of transposons on the loci that they disrupt. At the same time, these splice sites can potentially compromise expression of transposon sequences themselves. Thus, they argue that weak splice sites could be specifically favored as an adaptive compromise between these opposing forces.

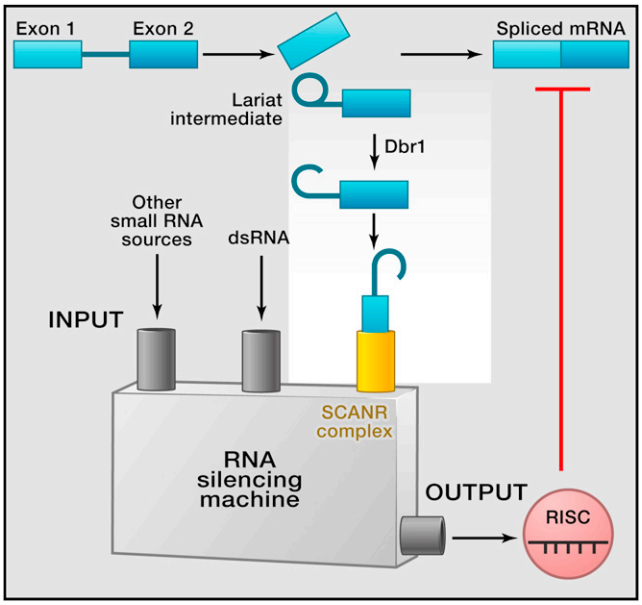

Figure 1. A Spliceosome-Based Source of Small RNAs Programs the Silencing Machinery in *C. neoformans*

The protein components of the RNA-silencing machinery require small RNAs derived from double-stranded RNAs and other sources to direct the complexes to specific targets. In *C. neoformans*, spliceosomes stall on suboptimal introns. These arrested complexes engage the SCANR complex, which uses the bound pre-mRNAs or splicing intermediates (likely after debranching by the Dbr1 enzyme) as templates for small-RNA biosynthesis. This novel pathway and the SCANR complex are highlighted. The spliceosome-derived small RNAs constitute a new input into the silencing machinery.

What of the suboptimally spliced transcripts in the host genome? Suboptimal introns are a hallmark of regulated splicing (Nilsen and Graveley, 2010) in part because their splicing can be readily modulated both positively and negatively. How can RNA silencing rely on the discrimination by the spliceosome without diminishing the spliceosome's regulatory potential on endogenous targets? Furthermore, could the splicing of particular transcripts be regulated under specific circumstances as a means to induce small-RNA silencing of those same genes? The discovery of the SCANR complex provides an exciting opportunity to answer these questions.

RNA-silencing pathways interface directly with many critical steps of eukaryotic gene expression and regulation, including chromatin assembly, transcription, mRNA stability, and translation. With a few tantalizing exceptions (e.g., Alló et al., 2009; Bayne et al., 2008; Ruby et al., 2007), pre-mRNA splicing has been conspicuously absent from this list. But this appears to be changing, thanks in part to the work of Dumesic et al. (2013). Indeed, a very recent and compelling phylogenetic connection has been identified between the splicing machinery and the RNAi apparatus (Tabach et al., 2013). siRNAs derived from stalled splicing complexes, as described by Dumesic et al. (2013), could represent an elegant exploitation of the spliceosome's highly honed aptitude for accuracy. Its discriminatory capacity, which normally supports the fidelity and regulation of endogenous gene expression, likely supports genome integrity as well. Even beyond this specific model, the discovery of the SCANR complex opens an intriguing door into the regulatory potential of the spliceosome and the RNA-silencing machinery and how they are intertwined and coordinated.

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